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De-novo mutations in patients with chronic ultra-refractory epilepsy with onset after age five years

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ABSTRACT

We set out to investigate whether a *de-novo* paradigm could explain genetic causes of chronic ultra-refractory epilepsy, with onset later than the typical age for the epileptic encephalopathies. We performed exome sequencing on nine adult patients with MRI-negative epilepsy and no preceding intellectual disability. All had an onset of seizures after five years old and had chronic ultra-refractory epilepsy defined here as having failed more than six anti-epileptic drugs and currently experiencing ≥4 disabling seizures per month. Parents were sequenced to identify *de-novo* mutations and these were assessed for likelihood of pathogenicity based on the American College of Medical Genetics and Genomics (ACMG) criteria. We confirmed the presence of functional and predicted-damaging *de-novo* mutations in 3/9 patients. One of these pathogenic *de-novo* mutations, in *DNM1L*, was previously reported in a patient with severe epilepsy and chronic pharmacoresistance adding to the evidence for *DNM1L* as an epilepsy gene. Exome sequencing is a successful strategy for identifying *de-novo* mutations in paediatric epileptic encephalopathies and rare neurological disorders. Our study demonstrates the potential benefit of considering ultra-refractory epilepsy patients with later onset for genetic testing. Identifying genetic mutations underpinning severe epilepsy of unknown aetiology may provide new insight into the underlying biology and offers the potential for therapeutic intervention in the form of precision medicine in older patients.

1. Introduction

Many patients with chronic refractory epilepsy develop seizures early in life (before age five), often as a manifestation of an underlying epileptic encephalopathy, along the West syndrome or Lennox-Gastaut syndrome phenotypic spectrum. A significant percentage of young children diagnosed with such an epileptic encephalopathy have a mutation in one of an increasing number of key genes involved in early brain development or in critical neuroregulatory functions such as synaptogenesis and cell cycle control (He et al., 2018). However, a significant number of patients develop refractory epilepsy and often secondary cognitive regression with onset after age five and after normal early neurodevelopment. Many of these patients can be described as having "ultra-refractory" epilepsy with a high seizure density and failure of many different AEDs to have a positive impact on seizure control.

An important paradigm in the genetic underpinnings of epilepsy is the pathological impact of *de-novo* mutations (Goldstein et al., 2013). Indeed, the importance of such mutations to epileptic encephalopathies has been appreciated for some time, but the application of next-generation sequencing is increasingly illustrating the nature and extent of the contribution (Allen et al., 2013). While some patients with epilepsy onset after age five will have an underlying structural brain epileptogenic lesion e.g. focal cortical dysplasia, many are non-lesional or 'MRI-negative'. The role of *de-novo* mutations in chronic refractory, later onset epilepsy is poorly understood. Understanding the genetic factors

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underlying epilepsy predisposition and treatment is vital for identifying much-needed diagnostic markers to help individualize patient care. We set out to determine the diagnostic yield of exome sequencing in a series of sporadic ultra-refractory epilepsy trios with onset greater than five years of age.

1.1. Patient data

We recruited subjects through epilepsy clinics associated with the EPIGEN Consortium at Beaumont and St. James' Hospitals, (Dublin) and Cork University Hospital (Cork) in Ireland (Sen et al., 2018). All study participants provided written, informed consent for genetic analysis. Local institutional review boards approved study protocols at each site. Our inclusion criteria were as follows: adult patients (> 18 years at recruitment) with active epilepsy, ≥ four seizures of any kind per month at recruitment (estimated from prior 12 months), failed ≥ six appropriate anti-epileptic drugs and an age of seizure onset > five years old. Subjects with an explanatory lesion on MRI, a preceding learning disability or first degree relative with epilepsy were excluded. Phenotype details were collected through clinical interactions and from hospital patient records.

2. Methods

We sequenced the exomes of nine probands and their unaffected parents. Five trios (UR001, UR007, UR008, UR011 and UR012) were sequenced at Columbia University Institute for Genomic Medicine (NY, USA) using the Roche NimbleGen SeqCap EZ Exome target enrichment platform on an Illumina HiSeq. A further four trios (UR002, UR003, UR004, UR005) were sequenced at Edinburgh Genomics (Scotland, UK) using the Ion Xpress Plus Fragment Library Kit enrichment platform on an Ion Personal Genome Machine. Paired-end or single read FASTQ files were aligned to human reference genome build GRCh37 with samtools and Burrows Wheeler Aligner (BWA) (Li and Durbin, 2009). Duplicate reads were checked and removed with Picard tools. Variant calling was performed with Genome Analysis Toolkit Haplotype Caller (GATK v3.0) according to the best practices pipeline (Depristo et al., 2011). Variants were phased with parental sequences to identify *de-novo* mutations assuming a prior *de-novo* rate of 1×10^{-6}. Variants were filtered for genotype quality (GQ > 20) and read depth (> 10x) and the final set of variants were annotated with ANNOVAR (Wang et al., 2010). We used the tools Sorting Intolerant from Tolerant (SIFT), Polyphen-2 and Combined Annotation Dependent Depletion (CADD) for *in silico* predictions of the functional effect of missense mutations.

We defined 'qualifying variants' as those that satisfied each of the following criteria: i) *de-novo* ii) minor allele frequency = 0 in gnomAD (http://gnomad.broadinstitute.org/), iii) missense or loss of function or frameshift or affecting a splice-site and iv) if missense, *in silico* predicted damaging by SIFT, Polyphen-2 or a CADD phred-scaled score > 10. In the event of a recessive disease, we also investigated homozygous variants with the same filtering criteria, but a minor allele frequency < 0.001 in gnomAD. We did not consider segregating reduced penetrance heterozygous variants since all cases had unaffected parents and no family history of seizure disorder. Qualifying variants were confirmed by Sanger sequencing. We followed ACMG guidelines to determine the pathogenicity of qualifying variants (Richards et al., 2015).

3. Results

A description of our case series is provided in Table 1.

We sequenced > 84% of the protein coding region at least 10 fold. Qualifying variants identified by exome sequencing are listed in Table 2.

We detected functional and predicted-damaging *de-novo* mutations in three probands. On analysis of the candidate variants using ACMG

Table 1
A phenotypic summary of the chronic refractory epilepsy cohort. AED: anti-epileptic drug, FLE: frontal lobe epilepsy, JME: juvenile myoclonic epilepsy, FS: febrile seizures, ID: intellectual disability, GTCS: generalised tonic-clonic seizures.

Proband (gender, onset age)	AEDs trialled	Epilepsy diagnosis	Seizure semiology	MRI	Comments
UR1 (M, 7)	16	FLE	Focal to bilateral tonic-clonic, focal with impaired awareness	Normal	Subdural haematoma, corpus callosotomy at age 30.
UR2 (F, 18)	18	Focal epilepsy, not otherwise specified	Focal with impaired awareness	Normal	Autism spectrum disorder.
UR3 (F, 11)	11	Multifocal epilepsy	Status epilepticus, focal with and without impaired awareness, myoclonus, focal to bilateral tonic-clonic	Cerebellar and cerebral atrophy	Normal development until episode of status epilepticus age 11. Profound learning difficulties and endocrine disorder.
UR4 (F, 7)	18	Multifocal epilepsy	GTCS, atonic, myoclonus	Normal	Abnormal motor coordination.
UR5 (F, 14)	14	JME	GTCS, myoclonus, absence	Normal	EEG bursts related to eye closure, no photosensitivity.
UR7 (F, 13)	8	TLE	Focal to bilateral tonic-clonic, focal with impaired awareness	Normal	Mild learning difficulties.
UR8 (F, 8)	14	FLE	Brief hypermotoric events	Normal	Normal development and no co-morbidities.
UR11 (F, 13)	10	Focal epilepsy, not otherwise specified	Focal with impaired awareness.	Normal	Mild learning difficulties, no other features to suggest underlying syndrome.
UR12 (M,7)	11	Left centro-parietal focal epilepsy	Focal with impaired awareness, early ocular movements	Normal	Normal development and no significant co-morbidities.

Table 2
Qualifying *de novo* variants detected in ultra-refractory probands. SNP positions are detailed in genome build GRCh37. MAF: minor allele frequency from gnomAD, ACMG: American College of Medical Genetics and Genomics classification, VUS: variant of unknown significance. PS: Strong pathogenic evidence, PM: Moderate pathogenic evidence, PP: Supporting pathogenic evidence.

Proband	Zygosity	Gene	Variant	SIFT	Polyphen-2	CADD	MAF	ACMG	ClinVar accession
UR1	*De-novo*	*OPLAH*	NM_017570.4:c.82G > A: p.(Gly28Arg)	Tolerated	Possibly damaging	22.7	0	VUS	SCV000852066
UR3	*De-novo*	*CEP55*	NM_018131.4:c.910A > T: p.(Ile304Leu)	Tolerated	Damaging	23.1	0	VUS	SCV000864021
UR3	*De-novo*	*DNM1L*	NM_012062.4:c.1207C > T:p.(Arg403Cys)	Deleterious	Damaging	33	0	Pathogenic (PS1, PS2, PM2, PP3, PP5)	SCV000864022
UR3	*De-novo*	*OSBPL7*	NM_145798.2:c.1078G > A:p.(Asp360Asn)	Tolerated	Damaging	23.7	0	VUS	SCV000864023

classification criteria, we could determine that UR3 had a pathogenic mutation in the gene *DNM1L*. There was insufficient evidence to assign pathogenicity to the variants detected in UR1. We did not observe any qualifying *de-novo* or recessive mutations in the probands UR2, UR4, UR5, UR7, UR8, UR10, UR11 or UR12.

4. Discussion

We present an exome sequencing case series of a severe, late-onset, sporadic refractory epilepsy. In all, we identified qualifying *de-novo* mutations in over half of our cases.

A single qualifying *de-novo* mutation in *OPLAH* was observed in proband UR1. OPLAH is associated with autosomal recessive 5-oxoproline deficiency however there is a single report of a heterozygous (S323R) paediatric patient with excessive urine pyroglutamic acid and seizure-onset at six weeks (Calpena et al., 2013). A follow-up urine organic acid metabolic screen of UR1 did not reveal excess pyroglutamate or any dysfunction of organic acid metabolism, therefore we concluded G28R to be a variant of unknown significance (VUS).

Three qualifying *de-novo* mutations were found in proband UR3. Two of these, D360N in *OSBPL7* and I304L in *CEP55,* were concluded to be variants of unknown significance. The third *de-novo* found in this proband is an R403C missense mutation in *DNM1L*. R40C is predicted to be highly deleterious, is absent from control populations, resides at a highly conserved amino acid and has been previously reported in the literature and in public databases (ClinVar variant ID: 214313). Therefore, it satisfies the criteria for pathogenicity according to ACMG guidelines. Of the previous report, the first describes an infant with refractory epilepsy and global developmental delay and experiencing generalised tonic-clonic seizures with episodes of status epilepticus beginning at one year old. This subject's EEG showed epileptiform activity in frontal and central regions yet MRI was normal (Vanstone et al., 2016). Meanwhile, two other carriers of this mutation had a delayed seizure onset, with episodes of status epilepticus following minor metabolic insult, preceding a rapidly progressing refractory encephalopathy with myoclonus and atrophy on MRI (Fahrner et al., 2016). We note the striking similarities in the phenotype and neurological decline between our proband and that of the cases in the Fahrner report. DNM1L is a member of the dynamin GTPase superfamily, is a mediator of mitochondrial and peroxisomal division and is highly expressed across brain tissues (Koch et al., 2003). Taken together, the evidence for pathogenicity of the R403C mutation in *DNM1L* cannot be disputed and highlights the importance of reporting candidate mutations in public databases.

5. Conclusion

In summary, from cohort of nine cases, we have identified a pathogenic mutation in one patient. This study is too small to draw conclusions regarding a diagnostic yield for this patient subgroup. Yet the *DNM1L* finding has demonstrated the potential benefit of considering severe refractory patients with onset later than the typical age for the epileptic encephalopathies for genetic testing. Further, as variant calling algorithms improve we hope to include copy number variant analysis from exome sequence data going forward which may in turn increase the number of positive findings. Later-onset encephalopathy is likely to be a composite of genetic disorders that present within the earlier age of onset of the epileptic encephalopathies, and other more unique disorders that present later in childhood, and perhaps beyond.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmg.2019.01.015.

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